Biotic interactions as drivers of algal origin and evolution

Brodie, Juliet; Ball, Steven G.; Bouget, François-Yves; Chan, Cheong Xin; De Clerck, Olivier; Cock, J. Mark

Published in:
New Phytologist

DOI:
10.1111/nph.14760

Publication date:
2017

Document Version
Peer reviewed version

Tansley review

Biotic interactions as drivers of algal origin and evolution

Juliet Brodie[1*], Steven G. Ball[2], François-Yves Bouget[3], Cheong Xin Chan[4], Olivier De Clerck[5], Mark Cock[6], Claire Gachon[7], Arthur R. Grossman[8], Thomas Mock[9], John Raven[10], Mahasweta Saha[11], Alison G. Smith[12], Assaf Vardi[13], Hwan Su Yoon[14], Debashish Bhattacharya[15*]

[1] Natural History Museum, Department of Life Sciences, London SW7 5BD, United Kingdom
[2] Université de Lille CNRS, UMR 8576 - UGSF- Unité de Glycobiologie Structurale et Fonctionnelle, F 59000 Lille, France
[3] University Pierre et Marie Curie, University of Paris VI, CNRS, Laboratoire d'Océanographie Microbienne, Observatoire Océanologique, F-66650, Banyuls-sur-Mer, France
[4] Institute for Molecular Bioscience and School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane QLD 4072, Australia
[5] Phycology Research Group, Ghent University, Krijgslaan 281, S8, 9000 Gent, Belgium
[6] CNRS, Sorbonne Université, UPMC University Paris 06, Algal Genetics Group, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, F-29688, Roscoff, France, 2Bezhin Rosko, 29250, Santec, France
[8] Department of Plant Biology, The Carnegie Institution for Science, Stanford, CA 94305, USA
[9] School of Environmental Sciences, University of East Anglia, Norwich NR47TJ, United Kingdom
[10] Division of Plant Sciences, University of Dundee at the James Hutton Institute, Dundee DD2 5DA, United Kingdom
[11] Helmholtz Center for Ocean Research, Kiel, 24105 Kiel, Germany
[12] Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, United Kingdom
[13] Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, 76100, Israel
[14] Department of Biological Sciences, Sungkyunkwan University, Suwon 440-746, Korea

[15] Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, NJ 08901, USA

[*] These authors contributed equally to this work.

Author for correspondence: *Debashish Bhattacharya*
Tel: +1 848 932 6218
Email: d.bhattacharya@rutgers.edu
Twitter handle: DebashB

Summary

Biotic interactions underlie life's diversity and are the lynchpin to understand its complexity and resilience within an ecological niche. Algal biologists have embraced this paradigm, and studies building on the explosive growth in omics and cell biology methods have facilitated in-depth analysis of non-model organisms and communities from a variety of ecosystems. In turn, these advances have enabled a major revision of our understanding of the origin and evolution of photosynthesis in eukaryotes, bacterial-algal interactions, control of massive algal blooms in the ocean, and the maintenance and degradation of coral reefs. Here we review some of the most exciting developments in the field of algal biotic interactions and identify challenges for the next generation of scientists. We foresee the development of an algal knowledgebase that integrates ecosystem-wide omics data and the development of molecular tools/resources to perform functional analyses of individuals in isolation and in populations. These assets will allow us to move beyond mechanistic studies of a single species towards understanding the interactions amongst algae and other organisms both in the laboratory and in the field.

Key words: algae, algal blooms, endosymbiosis, organellogenesis, genomics, holobiont, symbiome, trophic interactions.

I. Introduction

Algae are key primary producers in aquatic environments and represent several emerging genetic model systems (Armbrust *et al.*, 2004; Hopes *et al.*, 2016; Nymark *et al.*, 2016). They also play an increasingly important role in human nutrition (FAO, 2014). Algal photosynthesis provides about one-half of the oxygen that we breathe, and their genomes reveal the story of a tangled past that traverses the tree of life through the processes of endosymbiosis and horizontal gene transfer (HGT) (Price *et al.*, 2012; Cenci *et al.*, 2017). Biotic interactions between algae and other eukaryotes (e.g., (Worden *et al.*, 2015) are extremely widespread in aquatic and terrestrial ecosystems. The degree to which nature has experimented with these relationships is wide-ranging, including interactions among organisms that maintain a few functional associations, to those that have evolved a highly integrated suite of functions. In addition to the intracellular interactions described below, algae also engage in extracellular/surface interactions in the phycosphere, which is the ecologically and physiologically integrated neighborhood inhabited by the alga (Bell & Mitchell, 1972). Epibiosis (surface colonization of one organism [the basibiont] by other attached organisms [epibionts]) will not be covered in great detail here, but occurs on all immersed surfaces in the aquatic environment, including those of micro and macroalgae, and is of paramount importance in the marine environment (Wahl *et al.*, 2012). Epibiotic interactions (e.g., alga-alga, alga-bacterium, alga-virus [see below]) play key roles in nutrient acquisition and recycling, metabolic flux, energy flow and developmental processes. In parallel with herbivory, epibiosis represents one of the most important interactions that can determine the fate of an alga and has been shown to shape entire marine communities (Korpinen *et al.*, 2007).

In this review, we focus on research that has contributed some of the most exciting insights concerning the ways in which biotic interactions shape algal evolution and physiology. This perspective recognizes that “symbiomes” or “holobionts” are important targets of study to elucidate the overall capacity of genomes to interact with the environment. Here symbiome refers to co-localized and co-evolving (i.e., under selection) taxa comprising a given consortium, whereas holobiont includes all physically associated taxa regardless of the nature of the biotic interaction (Boucias *et al.*, 2013; Bordenstein & Theis, 2015; Douglas & Werren, 2016; Tripp *et al.*, 2017). This revolution in understanding integrative ecosystem function has largely been driven by the occurrence of technological advances in fields such as genomics, proteomics, and cell biology. It is clear, however, that we are on the cusp of far greater advances, as the concept

of the symbiome informs our experimental approaches. Below, we discuss prominent examples of algal biotic interactions that have been selected to illustrate the importance of these interactions in a broad range of contexts ranging from deep evolutionary time to processes of key relevance in the current context of global climate change. The review will begin with a discussion of the origin of photosynthetic organelles based on endosymbiosis, and will then look at algal interactions in the coral symbioses and the threat that climate change imposes on this association. Lastly we will examine the role of bacteria in algal biology, and the arms race associated with alga-virus interactions.

II. Endosymbiosis

1. Complex biotic interactions explain plastid origin

1.1. Primary endosymbiosis in Archaeplastida

Algae originated as a consequence of primary plastid endosymbiosis, a process in which a mitochondrion-containing, single-celled eukaryote engulfed and retained a cyanobacterium that eventually became the photosynthetic organelle or plastid (Cavalier-Smith, 1982; Bhattacharya *et al.*, 2004). The product of this ca. 1.6 billion year old endosymbiotic event (Yoon *et al.*, 2004) eventually split into the three primary plastid lineages, the red algae, the glaucophyte algae, and the green algae plus plants (together, the supergroup Archaeplastida) (Adl *et al.*, 2012; Price *et al.*, 2012). Algae from these groups were themselves frequently engulfed by other protists, giving rise to a rainbow of serially derived plastids distributed throughout the tree of life (Palmer, 2003; Gould *et al.*, 2008) (Fig. 1a). The process of primary plastid capture has sometimes been depicted as a 'hungry' single-celled eukaryote engulfing a prokaryote followed by the subsequent evolution of a functional organelle. This portrayal begs the obvious question: if the process is so simple, then why has the event been so rare given that oceans and lakes are replete with phagotrophic protists that have been feeding on prokaryote prey for hundreds of millions of years? In fact, there are only two bona fide primary endosymbioses known that gave rise to widespread organelles over the long history of eukaryotes; the event from which all plastids originated, as explained above, and a prior event that led to the evolution of mitochondria. Other more taxonomically limited cases of organelle origin are associated with the photosynthetic amoeba lineage *Paulinella* (see below), the non-photosynthetic organelle of the trypanosomatids (Kostygov *et al.*, 2016; Morales *et al.*, 2016), and nitrogen fixing spheroid

bodies in the rhopalodiacean diatoms (Nakayama *et al.*, 2014; Zehr *et al.*, 2016). The rarity of primary endosymbiosis has fascinated scientists for many years and is usually attributed to the extensive innovations required for organelle establishment. These include: a) events that lead to the protection of the nascent endosymbiont from host digestion; b) tailoring of processes critical for the exchange of metabolites between the endosymbiont and host cell (Facchinelli & Weber, 2011); c) the origin of an import system to move cytosolic proteins into the nascent organelle (Schleiff & Becker, 2011); d) foreign gene acquisition through HGT and the integration of the HGT-derived protein products into both host and newly developing organelle pathways (Cavalier-Smith, 2002; Karkar *et al.*, 2015); and e) movement of genes from the organelle to the host nucleus to escape Muller's ratchet, i.e., accumulation of mutations in non-recombining genomes (Felsenstein, 1974). Processes that would exacerbate the impact of Muller's ratchet and make relocation of genes from the organelle to the nuclear genome more imperative are the mutagenic effect of damaging reactive oxygen species (ROS) produced as a consequence of photosynthesis in the organelle (van Creveld *et al.*, 2015), and as yet unexplained processes associated with greater damage of DNA in organelles than in their aerobic bacterial ancestors (Raven, 2015). Explanations for why organelle genomes are retained include coordinated synthesis of complexes assembled in the organelle, and the regulation of transcriptional and post-transcriptional processes by the organelle redox state (van Creveld *et al.*, 2015).

The most critical innovation listed above, is the first, namely how a captured bacterial cell evades digestion by the host during the initial stages of plastid evolution. A potential answer to this question comes from recent work exploring the evolution of mitochondria. Current mitochondrial gene phylogenies indicate that this organelle originated from anciently diverged environmental Rickettsiales-like pathogens with relatively large gene inventories (Wang & Wu, 2015; Ball *et al.*, 2016c) whose descendants are now often found in association with protists (Martijn *et al.*, 2015). However, these taxa are distinct from the highly specialized animal parasites with streamlined genomes, such as the typhus agent *Rickettsia prowazekii* (Zomorodipour & Andersson, 1999), that were initially proposed as the alpha-proteobacterial candidates based on limited data that was collected over ten years ago (Emelyanov, 2003). The host of this mitochondrial endosymbiosis was likely to be a member of the recently discovered archaeal 'Asgard' superphylum (including the Lokiarchaeota and Heimdallarchaeota), which is the most closely related prokaryote to the eukaryote nuclear lineage (Spang *et al.*, 2015;

Zaremba-Niedzwiedzka *et al.*, 2017). Therefore, the increasingly widely accepted view is that an Asgard-like cell was infected by a relatively gene-rich Rickettsiales-like pathogen, thus laying the foundation for mitochondrial endosymbiosis and eukaryogenesis. By virtue of their existing ability to thrive in the intracellular environment, the ancestors of mitochondria were pre-adapted to switch from pathogenesis to endosymbiosis. These cells had evolved efficient solutions to deal with host innate immunity due to millions of years of coevolution with the Asgard lineage. These findings suggest that, to become a successful proto-endosymbiont, the invading cell needs to evade host defenses, which is more likely to be achieved by an intracellular pathogen adapted to the cytosolic lifestyle (Ball *et al.*, 2016b; Ball *et al.*, 2016c; Cenci *et al.*, 2017).

Application of this concept to the origin of plastids requires some modification because extant cyanobacteria are not intracellular pathogens and lack the inherent capacity to evade host defenses. We suggest two possible explanations for cyanobacterial survival. First, the Archaeplastida host of this endosymbiosis may have developed mutations that reduced the efficacy of its lytic/phagocytic functions. This provided the cyanobacterium sufficient residence time within a host food vacuole to evolve a character(s) beneficial to the host (e.g., secretion of fixed carbon or reduced nitrogen compounds), which allowed the establishment and spread of a founder population. This scenario is more likely to have occurred in oligotrophic waters, which lacked abundant prey. An alternative explanation is that the cyanobacterium was protected by a third 'player' that could withstand host defenses. This latter idea receives support from the finding that there are several dozen genes of chlamydial origin present in the nuclear genome of algae and plants (Huang & Gogarten, 2007; Becker *et al.*, 2008). Phylogenetic data suggest that these genes are from environmental strains with relatively large genomes, such as those that infect *Acanthamoeba*, and not the highly reduced human pathogens. In addition, many of the products of these nucleus-encoded genes are plastid targeted and perform specialized functions not associated with cyanobacteria (Huang & Gogarten, 2007; Moustafa *et al.*, 2008). These observations have led to the "ménage à trois" hypothesis (MATH) to explain the origin of plastids. In this scenario a Chlamydiales ancestor evolved from a pathogenic to symbiotic lifestyle, protecting the cyanobacterium in its inclusion vesicle (Ball *et al.*, 2013; Cenci *et al.*, 2017). Although the MATH remains controversial due largely to issues associated with 'deep time' gene phylogenies and the unresolved role of HGT in eukaryote evolution (Dagan *et al.*, 2013; Ball *et al.*, 2016a), its complexity reflects well-established biotic interactions. As

illustrated in Fig. 1b, it predicts that an elementary body (chlamydial infectious particle) escapes host defenses by remodeling the phagocytic membrane and by secreting chlamydial effector proteins that enable bacterial specific metabolites of photosynthesis such as ADP-glucose to enter the host cytosolic glycogen stores. Both glaucophytes and red algae store carbohydrates in their cytosol suggesting that the glycogen/starch pool may have provided an opportunity to buffer the unsynchronized demand and supply of carbon of the cyanobiont and its host. Several observations support this idea: a) enzymes involved in manipulating host carbohydrate metabolism are pathogen effectors secreted by the type-III secretion system (Gehre *et al.*, 2016); b) pathogenic Chlamydiae synthesize extracellular storage carbohydrates within parasitophorous vacuoles using analogous nucleotide-sugars and nucleotide-sugar transporters (Gehre *et al.*, 2016); c) nucleotide-sugar transporters of host origin are evolutionary ancestors of plastid carbon exporters in red and green algae, as well as in plastids of secondary or tertiary endosymbiotic origin (Moog *et al.*, 2015); and d) analysis of the tryptophan biosynthesis pathway in Archaeplastida shows that one-half (4/8) of the genes encoding proteins in this pathway are putatively of chlamydial origin, as are the *E. coli tyr/mtr* (tyrosine/tryptophan) transporter genes (Cenci *et al.*, 2016; Cenci *et al.*, 2017).

Tryptophan starvation may have been a mechanism used by the host of the primary plastid to combat chlamydial infection (Bonner *et al.*, 2014). Tryptophan biosynthesis is by far the most costly amino acid for cells to synthesize. In comparison to the eukaryotic host and the cyanobiont, sensitivity of the chlamydial symbiont to tryptophan starvation would have been exacerbated by the energy requirements for its synthesis (Bonner *et al.*, 2014). This biotic interaction would therefore have selected for movement of the chlamydial *trp* operon to the cyanobacterial endosymbiont genome to ensure high levels of gene expression. Cenci *et al*. (2016) posit that the chlamydial *trp* operon transfer occurred via conjugation during co-localization of chlamydial and cyanobacterial cells in inclusion vesicles. At a later time, some *trp* genes were moved to the Archaeplastida nuclear genome by endosymbiotic gene transfer (EGT) (Martin & Herrmann, 1998) from the cyanobacterial plastid forerunner. The MATH is reinforced not only by functional considerations, but also gene numbers. Chlamydiae HGTs are not scattered randomly among the organisms of the tree of life, but rather, an outsize contribution (ca. 30-50 genes, depending on the lineage being studied) is found when compared to other non-cyanobacterial prokaryotic gene acquisitions in Archaeplastida nuclear genomes (Huang &

Gogarten, 2007; Deschamps, 2014). In addition, analysis of the plastid proteome shows that despite having >50-fold more proteobacterial than chlamydial sequences in current genome databases (e.g., National Center for Biotechnology Information), Proteobacteria and Chlamydiae genes represent the largest contribution to plastid functions (46 and 24 genes, respectively, in *Arabidopsis thaliana)*, with only 13 from alpha-Proteobacteria (Qiu *et al.*, 2013). The MATH provides a testable model that can be used to study the steps that led to plastid origin. Beyond its specific predictions (Ball *et al.*, 2013; Cenci *et al.*, 2017), this theory highlights the complexity of biotic interactions that underlie endosymbiosis. In the future, the aim should be to develop systems in the laboratory to study the processes underlying endosymbiosis so that we can move beyond trees and diagrams, to allow experimental elucidation of mechanisms underlying organellogenesis.

1.2. Origin of the *Paulinella* chromatophore

The concept of multiple microbes contributing to plastid evolution in the Archaeplastida, may also explain the maintenance and evolution of the plastid (termed the chromatophore) in *Paulinella chromatophora* (Marin *et al.*, 2005; Nowack *et al.*, 2008; Yoon *et al.*, 2009). In this case, there is currently no evidence for chlamydial-facilitated organelle origin. However, over 200 bacterium-derived HGTs have been found in the nuclear genome of this species that complement gene losses from the chromatophore genome. Specifically, many missing components of critical endosymbiont pathways, such as for amino acid and peptidoglycan biosynthesis and DNA replication, have been compensated for by the acquisition of a variety of prokaryotic donor genes via HGT (Nowack *et al.*, 2016). Access to these foreign genes was likely facilitated by phagotrophic uptake of bacteria by the host amoeba, followed by HGT of DNA to the amoeba nuclear genome. Once activated, nucleus-encoded gene products were relocated to the chromatophore, possibly by trafficking through the secretory system, where they could replace components of the pathways encoded on the chromatophore genome (Nowack & Grossman, 2012). It should be stressed that a response to Muller's ratchet acting on the chromatophore genome (leading to genome reduction) in *P. chromatophora* is certainly expected, but surprisingly, it is not primarily EGT and rerouting of host proteins in this relatively 'young' endosymbiosis (i.e., 90-140 million years old; Delaye *et al.*, 2016) that facilitates this process, but rather, repurposing of environmental DNA as a result of biotic interactions.

2. Complex biotic interactions explain the symbiosis between algae and corals

2.1. Maintenance of the symbiosis

Corals are the structural and trophic foundation of coral reefs, which support about 30% of all described marine species (Wilkinson, 2004). Critically, reef-building corals are a symbiosis between the coral animal *per se* and photosynthetic dinoflagellates in the genus *Symbiodinium* (Figs. 2a, 2b3). *Symbiodinium* are also key algal symbionts in a wide range of coral reef animals, including sea anemone, sponges, jellyfish, and clams. The coral-*Symbiodinium* association is one of relaxed specificity: individual corals can harbor alternative and multiple symbiont types simultaneously, and a *Symbiodinium* type may associate with a range of coral hosts (Silverstein *et al.*, 2012). Upon acquisition of *Symbiodinium* by host gastrodermal cells (within which the algal cells reside), the *Symbiodinium* are physically separated from the cytoplasm by a host-derived vacuole known as the symbiosome (Roth *et al.*, 1988). Exposure to competent *Symbiodinium* cells triggers an initial stress response in the coral *Acropora digitifera*, resulting in transient suppression of protein synthesis and mitochondrial metabolism (Mohamed *et al.*, 2016). This finding supports the hypothesis that the symbiosome is a phagosome that has undergone early arrest (Shinzato *et al.*, 2011; Mohamed *et al.*, 2016).

Coral reefs thrive in nutrient-poor waters. In return for shelter (e.g., from ultraviolet radiation, predation), *Symbiodinium* photosynthesis may provide >90% of the fixed carbon requirement (Muscatine & Porter, 1977) of the hosts. A critical limitation of photosynthesis is access to dissolved inorganic carbon. Since they have no direct access to ambient seawater, *Symbiodinium* cells depend on the host for delivery of inorganic carbon (C_i; CO_2 or HCO_3^-) (see Fig. 2c). When net photosynthesis takes place some C_i is generated via respiration, but in corals the predominant C_i supply to photosynthesis is its accumulation within host tissue from external sources (Shinzato *et al.*, 2011). The concentration of C_i in the host tissue can be ~70-fold that of seawater, which represents a steeper gradient than is observed for most organisms that use a carbon concentrating mechanism (CCM) (Shinzato *et al.*, 2011). C_i accumulation by the host could also be related to the existence of an acidified space between the algal cell and the symbiosome membrane, and the presence of an uncharacterized HCO_3^- transporter in the symbiosome membrane and carbonic anhydrase activity in the acidified space. The host also

appears to have an active role in regulating photosynthesis in the symbionts (Barott *et al.*, 2015; Bhattacharya *et al.*, 2016).

The algae of the holobionts also accumulate C_i (Walker *et al.*, 1980; Barott *et al.*, 2015). This is likely related to the fact that dinoflagellates such as *Symbiodinium* have Form II ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO), which shows low CO_2:O_2 selectivity and, probably, a low affinity for CO_2 (Leggat *et al.*, 2000). Other symbioses have different roles for the animal in C_i supply to *Symbiodinium*; e.g., in tridacnid giant clams, where the symbionts are extracellular, the haemolymph is the immediate source of C_i. The C_i concentration in the haemolymph in the light is lower than that in seawater (Muscatine & Porter, 1977), thus there is no evidence for accumulation of C_i to higher concentrations than in seawater during influx through the gill epithelium. In this case, the accumulation of C_i by the photobiont presumably plays an even more vital role in algal primary production.

The symbiotic relationship between *Symbiodinium* and its coral hosts determines not only the rate of coral-reef growth (calcium carbonate deposition), but also how corals respond to environmental stress (Voolstra *et al.*, 2015). A modest episodic period of increased temperature of the ocean surface (e.g., a few days at 1-2°C above the mean summer minimum) can set off a cascade of photoinhibition, the decoupling of carbon flow between the symbiont and host (breakdown of symbiosis), oxidative damage, and physical loss of symbiont cells (Wooldridge, 2013). This process, known as coral bleaching, leaves the coral host at risk for starvation, disease, and death unless the symbiosis is soon re-established (Hoegh-Guldberg, 1999). In this way, algae are essential for survival and maintenance of coral reef ecosystems. The impact of current environmental change on the health of the symbiotic association is particularly alarming, especially in recent years. For example, >90% of the 911 reefs surveyed in 2015-2016 at the Great Barrier Reef (the world's largest continuous reef system) showed signs of severe bleaching (Albright *et al.*, 2016).

2.2. Omics perspective on the coral symbiosis

The cellular and molecular processes of symbiosis that are actively being explored include recognition, capture of the symbiont in the symbiosome, proliferation of symbionts in host tissue, loss of symbionts from the host tissue, and metabolic exchange and nutrient trafficking between *Symbiodinium* and the host across multiple membranes. There is much to be learned about these

topics from a broader genomic and molecular evolutionary perspective. *Symbiodinium* is classified into nine clades based on phylogenetic markers, although they represent a highly divergent group of dinoflagellate species (LaJeunesse *et al.*, 2005; Wham & LaJeunesse, 2016) and may include >100 species capable of forming symbiotic associations with corals. Hurdles in studying genomic and molecular aspects of the coral system include difficulties associated with the establishment of axenic cultures of the various *Symbiodinium* types, their slow growth, and the size and complexity of their genomes. The dinoflagellate nuclear genome can be massive, up to 250 Gbp in size (LaJeunesse *et al.*, 2005; Lin, 2011), and exhibits unusual features including non-canonical nucleotides, atypical intron-exon splice signals (Lin, 2011), and RNAs that are trans-spliced (Zhang *et al.*, 2007). RNA editing of transcripts has been described in mitochondrial and plastid genomes (Lin, 2011; Jackson & Waller, 2013; Mungpakdee *et al.*, 2014), whilst the plastid genome is comprised of distinct DNA minicircles, each containing a gene, a few genes, or in some cases, no genes (Zhang *et al.*, 1999; Howe *et al.*, 2008). The nuclear genomic features are set against a backdrop of gene or genome-fragment duplications, and abundant non-coding repetitive elements (McEwan *et al.*, 2008; Shoguchi *et al.*, 2013). Three genome sequences of *Symbiodinium* from distinct clades were recently published (Shoguchi *et al.*, 2013; Lin *et al.*, 2015; Aranda *et al.*, 2016), and their estimated genome sizes of 1.1-1.5 Gbp are smaller than the earlier estimates of 3-5 Gbp (LaJeunesse *et al.*, 2005). In addition, these genomes share little sequence similarity; i.e., <1% of total sequenced reads from *S. kawagutii* mapped onto the genome assembly of *S. minutum*, and vice versa (Lin *et al.*, 2015). These results indicate a high level of genome divergence among distinct *Symbiodinium* clades.

Further complexity in corals comes from a three-way functional complementarity between the coral host, the dinoflagellate, and the associated microbiome of bacteria and viruses (Ziegler *et al.*, 2017). For example, the incomplete cysteine biosynthesis pathway in the coral *Acropora digitifera* (Shinzato *et al.*, 2011; Shinzato *et al.*, 2014) is compensated for by *Symbiodinium* (Shoguchi *et al.*, 2013; Lin *et al.*, 2015), whereas bacteria likely play a key role in regulating the availability of nitrogen to the coral host and algae and in resistance to thermal stress (Radecker *et al.*, 2015; Ziegler *et al.*, 2017). Nevertheless, given the diversity of *Symbiodinium* species, a 'one-reference-genome-fits-all' assumption will not be possible for studying the coral-dinoflagellate symbiosis and interactions, and additional genome data from the different species types/clades will be necessary. An effective approach would be to integrate

multi-omics data from the coral and the associated *Symbiodinium* and microbiome; i.e. the holobiont (Bordenstein & Theis, 2015), to tease apart the individual contributions of each component in sustaining a healthy holobiont. Availability of additional data from free-living dinoflagellates will help address key questions including the evolutionary events and functional innovations that lead to the transition from a free-living to a symbiotic lifestyle. At the same time, tractable lab model systems are being developed (Shapiro *et al.*, 2016) that will enable the study of cellular mechanisms that underlie the response to elevated temperature and pathogens. Findings from such studies will inform strategies for conservation of and risk mitigation for reef ecosystems.

III. Biotic interactions within the phycosphere

3.1 Alga-bacterium biotic interactions

Interactions between algae and bacteria are likely to be universal in the environment. Many notable examples are species specific, such as the green seaweed *Ulva mutabilis,* which relies on different bacterial strains for successful morphogenesis (Spoerner *et al.*, 2012). In the laboratory, rather than forming the typical blade- or tube-like morphology, axenic gametes of *U. mutabilis* develop into callus-like aggregates of undifferentiated cells with abnormal cell walls. These findings suggest the existence of chemical signaling between bacteria and the alga, and potentially, complementarity of metabolic pathways. Similar interactions have also been found between the bacterium *Sulfitobacter pseudonitzschiae* and the diatom *Pseudo-nitzschia multiseries* (Amin *et al.*, 2015), and bacteria have been shown to facilitate acclimation of the brown seaweed *Ectocarpus siliculosis* to a freshwater environment (Dittami *et al.*, 2016). Another striking example of this phenomenon is the ‘Jekyll-and-Hyde’ (named by the authors) relationship between the roseobacter *Phaeobacter gallaeciensis*, a biofilm forming symbiont of the bloom-forming haptophyte alga *Emiliania huxleyi* (Seyedsayamdost *et al.*, 2011). Under normal growth conditions *P. gallaeciensis* secretes antibiotics and growth phytohormones (e.g. the auxin indole-3-acetic acid) that appear to benefit the alga. However, as the algal population ages, the bacteria shift their small molecule biosynthesis pathways to the production of algaecides, and act as an *E. huxleyi* pathogen (Seyedsayamdost *et al.*, 2011; Segev *et al.*, 2016). A different type of biotic interaction involves capture and ‘farming’ of the cryptophyte alga

Teleaulax amphioxeia by its host ciliate, *Mesodinium rubrum*, to extract nutrients from the captured, intact alga (Qiu *et al.*, 2016).

More general interactions are seen with bacteria that play a key role in providing micronutrients to algae. Examples are essential organic compounds such as thiamine (vitamin B_1) and cobalamin (vitamin B_{12}). These compounds are required as enzyme cofactors, but many phytoplankton species are unable to synthesize them. Only prokaryotes (and only then, a subset of both Eubacteria and Archaea) can synthesize cobalamin *de novo* (Warren *et al.*, 2002), and levels free in the aquatic environment are generally too low to support algal growth (Sañudo-Wilhelmy *et al.*, 2012). Direct provision of the vitamin from bacteria to algae has been demonstrated in the laboratory (Croft *et al.*, 2005; Wagner-Döbler *et al.*, 2010; Kazamia & Smith, 2014; Durham *et al.*, 2015) and evidence that similar exchanges occur in the natural environment comes from correlations observed between the presence of B_{12}-producing bacteria and algal blooms (Gobler *et al.*, 2007; Bertrand *et al.*, 2015). There is still specificity in this interaction, however, demonstrated by the fact that, whilst cyanobacteria are B_{12} producers, they make a variant known as pseudocobalamin which is considerably less bioavailable to eukaryotic algae than cobalamin, the variant produced by many heterotrophic bacteria (Helliwell *et al.*, 2016). Thus provision of photosynthate from the algae may provide the signal to attract and retain cobalamin-producers within the phycosphere. As well as B_{12}, recent studies have demonstrated that bacteria can also provide either thiamine (vitamin B_1) or its precursors to phytoplankton (McRose *et al.*, 2014; Paerl *et al.*, 2015), and because these organic micronutrients are often limiting in the ocean (Sañudo-Wilhelmy *et al.*, 2012), thiamine-producing bacteria could potentially regulate phytoplankton blooms.

The specificity and extent of such algal-bacterial interactions in the natural environment remain to be determined however. One exciting development that will enable better understanding of the diverse and multifaceted ways in which algal cells interact with their biotic and abiotic environments is the explosion of metagenomics and metatranscriptomics information that is being produced by projects such as the TARA Oceans Expedition (Bork *et al.*, 2015). Current analyses of the 'interactome' in the photic zone have revealed novel partnerships and unexpected factors controlling community structure (Lima-Mendez *et al.*, 2015). Together with mechanistic examinations of algal physiology and biochemistry in laboratory conditions (e.g. (Durham *et al.*, 2015), these omics-enabled analyses will fundamentally change our views of

how algae sense and survive in the current world, and how resilient they may be to fluctuating conditions wrought by climate change.

3.2. Host-virus arms race during algal blooms

3.2.1. Viral control of algal blooms

Many algal species exhibit the phenomenon of 'blooms', for example 'red tides', where there is a massive increase in cell numbers over a short period, frequently as a result of changing environmental conditions, such as agricultural run-off or ocean upwelling. In some cases, these can pose threats to human health (so-called harmful algal blooms; HABs) due to the toxins that are produced by the algae and/or associated bacteria (Petitpas *et al.*, 2014). Such blooms are ephemeral events of exceptionally high primary productivity that regulate the flux of nutrients and metabolites across aquatic food webs. These large-scale events also contribute to global net primary production, one-half of which is provided by oceanic phytoplankton (Behrenfeld *et al.*, 2006). Several key biotic interactions can control the extent and fate of phytoplankton blooms in the ocean, among them top-down regulation by grazers, interactions with algicidal bacteria, and viral infection (Bidle, 2015). Viruses play a key role in this process because they infect many marine algal species, such as the major 'brown tide' alga *Aureococcus anophagefferens* (Moniruzzaman *et al.*, 2016), resulting in cessation of phytoplankton blooms. Viruses are the most abundant biological entities in the marine environment and are considered to be major ecological, evolutionary and biogeochemical drivers of marine microbial life (Suttle, 2007). Moreover, they enhance the diversity and composition of the microbial communities by facilitating HGT among their hosts.

Recent reports have highlighted a novel inventory of auxiliary metabolic genes found in the genomes of marine viruses that were previously thought to be restricted to the genomes of their hosts (Enav *et al.*, 2014; Rosenwasser *et al.*, 2016) with functions including photosynthesis, the pentose phosphate pathway, phosphate regulation, sulfur metabolism, polysaccharide synthesis, sphingolipid metabolism, and DNA/RNA processing. These genes can expand metabolic capabilities within the infected phototrophs and affect the flux of metabolites and infochemicals to the phycosphere. Viruses infecting terrestrial plants are typically small RNA viruses that encode a few genes and therefore their life cycle is tightly integrated with and

dependent on the cellular processes of their host plants (Roossinck, 1997). In contrast, viruses that infect eukaryotic algae can have a high burst size (i.e., number of viruses released from each infected cell), and have genomes of 160 to 560 kbp that encode up to 600 proteins (Wilson *et al.*, 2009). Thus, these viruses require substantial resources such as fatty acids, amino acids, nucleotides, and energy to facilitate replication and assembly. Nevertheless, there is still no fundamental understanding of how such large viruses rewire the metabolism of their photosynthetic host to support their unique life cycle.

Although the ecological importance of host-virus interactions is well recognized, the ability to assess their functional/ecological impact is limited to current approaches that focus mainly on quantification of viral abundance, gene content and diversity (Brum & Sullivan, 2015). Developing laboratory-based model systems for ecologically relevant algal-virus interactions, coupled with a molecular toolbox and genomic and post-genomics resources have deepened our mechanistic understanding of these interactions and their ecological impact (Fig. 3) (Read *et al.*, 2013).

3.2.2. *Emiliania huxleyi*-EhV- an important host-pathogen model system

The cosmopolitan coccolithophore *E. huxleyi* is a unicellular alga that forms massive oceanic blooms covering thousands of square kilometers (Tyrrell & Merico, 2004). The intricate calcite exoskeleton of *E. huxleyi* accounts for approximately one third of total marine $CaCO_3$ production (Monteiro *et al.*, 2016). *E. huxleyi* is also a major producer of dimethyl sulfide (DMS), a bioactive gas with a significant climate-regulating role that enhances cloud formation (Alcolombri *et al.*, 2015). Therefore, biotic interactions that regulate the fate of these blooms play a profound role in determining atmospheric conditions and nutrient cycling in the ocean. Annual *E. huxleyi* spring blooms are frequently terminated by infection with a specific large dsDNA virus (EhV) (Schroeder *et al.*, 2002) that belongs to the Coccolithoviruses group within the monophyletic Phycodnaviridae, a family of nucleocytoplasmic large DNA viruses. This model host-virus interaction spans more than 10 orders of spatial magnitude, from the individual cell (~10^{-6} m) to mesoscale oceanic eddies (~10^{5} m) (Lehahn *et al.*, 2014). The system is physiologically well characterized and has the great advantage of a wealth of genomic information from the alga (Read *et al.*, 2013) and from specific viral strains with different degrees of susceptibility to viral infection. Genome analysis of EhV revealed a cluster of putative

sphingolipid biosynthetic genes (Wilson *et al.*, 2005). Production of glycosphingolipids is strongly induced during viral infection. These lipids are major constituents of EhV membranes and can induce host programmed cell death (PCD) during lytic infection in cultures and during natural blooms (Vardi *et al.*, 2012). Indeed, during lytic infection, EhV triggers hallmark PCD responses, including production of ROS (Vardi *et al.*, 2012; Sheyn *et al.*, 2016), induction of caspase activity, metacaspase expression and compromised membrane integrity (Bidle *et al.*, 2007). Viral infection also induced remodeling of the host antioxidant gene network and redox metabolism through co-induction of glutathione and H_2O_2 synthesis, both essential for successful viral replication (Sheyn *et al.*, 2016). Viral infection "engineers" sphingolipid metabolism of the host by causing down-regulation of host sphingolipid biosynthesis genes while the viral genes are highly up-regulated (Rosenwasser *et al.*, 2014), resulting in altered substrate specificity of serine palmitoyl-CoA transferase activity (Ziv *et al.*, 2016). The viral enzymes have different substrate specificities from those of the host and regulate the production of virus-specific glycosphingolipids composed of unusual hydroxylated C17 sphingoid-bases (t17:0) (Ziv *et al.*, 2016). These virus-specific sphingolipids are essential for assembly and infectivity by the virion. Combined transcriptomic and metabolomic analyses over the course of an *E. huxleyi* viral infection revealed major, rapid transcriptome remodeling that elicited elevated de novo fatty acid synthesis to support viral assembly and a high demand for viral internal lipid membranes (Rosenwasser *et al.*, 2014). Remodeling of lipid metabolism was mediated by accumulation of distinct lipid droplets containing highly saturated triacylglycerols (TAGs) (Malitsky *et al.*, 2016). Stored TAGs may serve as energy and lipid reservoirs that are catabolized for viral assembly during later stages of infection.

These approaches, which involved rigorous quantification of the rewired metabolism during algal-virus interactions have provided fundamental insights into the strategies employed during their biochemical "arms race". Identification of specific metabolites synthesized during these interactions may yield biomarkers for sensitive detection of active viral infection in the marine environment (Vardi *et al.*, 2009).

IV. Future prospects

As described in this review, there has been significant progress in studies of the algal symbiome that stress the primacy of biotic factors in algal growth and productivity in the environment.

These analyses have provided significant mechanistic insight into emerging systems across the algal tree of life. Nevertheless, there still remain many gaps in our knowledge and approaches. For example most studies at the functional level have focused on "pairs" such as bacteria/microalgae or viruses/microalgae, whereas these are likely to be much more complex in the natural environment. Similarly, whilst studies of microbial communities during annually reoccurring phytoplankton blooms provide clues about microalgae/bacteria interactions at the community level and in relation to changing environmental conditions, including those driven by global change (e.g. (Needham & Fuhrman, 2016), few address specific interactions. This is important because short-term fluctuations of environmental parameters (e.g., diurnal fluctuations) may be buffered by biotic interactions and are therefore invisible to the investigator, which would lead to the conclusion that they are not important, even though they might have an impact over a longer time scale. Furthermore, most studies do not look beyond correlations based on co-occurrence networks, which, while providing useful preliminary data on who interacts with whom, do not provide insights into the biological processes that orchestrate these interactions.

To tackle these challenges, future studies should include detailed biochemical analyses of metabolites both in environmental samples *in situ* and under controlled laboratory conditions, using either natural or synthetic communities. The combined analyses of natural and synthetic communities and the use of microbial mutants that impact specific pathways will help determine activities associated with ecosystem function. Genome editing applied to model microalgae and bacteria in combination with biochemical analyses of processes that govern their interactions will provide a step change in understanding how significant these interactions are in relation to abiotic drivers of biological diversity such as temperature, nutrients, seasonality and solar irradiance. By studying communities across global-scale environmental gradients such as coastal/open sea, surface/deep ocean, or polar/tropics, it should be possible to identify commonalities between taxonomically distinct, yet functionally equivalent communities.

Finally, metagenomic data is of vital importance to this field, but need to be combined with functional studies. We are now presented with an overwhelming amount of genomics and meta data and the time has come to start ferreting out the biological 'meaning' of this information using algal model systems, genetic tools, and functional genomics to understand gene function and cellular mechanism and connect these insights with in-depth studies of

physiology, metabolism, and life cycle phenotypes. Adding the new dimension of single cell analysis is another emerging area that will likely fundamentally chmage how we interpret algal diversity, behavior, and acclimation strategies. With these integrative approaches, we may even be able to provide key insights into how global change not only impacts the diversity of specific taxa but the complex interacting communities of species in the ocean that underpin marine ecosystem services responsible for the health and well-being of human societies.

Acknowledgements

This manuscript is an outcome of a symposium hosted in June 2016 by The Royal Society entitled, “Into the genome: advances in the world of algal genomics,” in Chicheley Hall, Buckinghamshire, United Kingdom. The symposium organizers, J.B. and D.B. are grateful to the Royal Society for supporting this event. The University of Dundee is a registered Scottish charity, No 015096. We thank Dr. Jean-Baptiste Raina at the University of Technology, Sydney, Australia who generously provided the *Symbiodinium* images.

References

Adl SM, Simpson AG, Lane CE, Lukeš J, Bass D, Bowser SS, Brown MW, Burki F, Dunthorn M, Hampl V et al. 2012. The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology* **59**: 429-493.

Albright R, Anthony KRN, Baird M, Beeden R, Byrne M, Collier C, Dove S, Fabricius K, Hoegh-Guldberg O, Kelly RP et al. 2016. Ocean acidification: linking science to management solutions using the Great Barrier Reef as a case study. *Journal of Environmental Management* **182**: 641-650.

Alcolombri U, Ben-Dor S, Feldmesser E, Levin Y, Tawfik DS, Vardi A. 2015. Identification of the algal dimethyl sulfide-releasing enzyme: A missing link in the marine sulfur cycle. *Science* **348**: 1466-1469.

Amin SA, Hmelo LR, van Tol HM, Durham BP, Carlson LT, Heal KR, Morales RL, Berthiaume CT, Parker MS, Djunaedi B et al. 2015. Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* **522**: 98-101.

Aranda M, Li Y, Liew YJ, Baumgarten S, Simakov O, Wilson MC, Piel J, Ashoor H, Bougouffa S, Bajic VB et al. 2016. Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. *Scientific Reports* **6**: 39734.

Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH, Zhou S, Allen AE, Apt KE, Bechner M et al. 2004. The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science* **306**: 79-86.

Ball SG, Bhattacharya D, Qiu H, Weber AP. 2016a. Commentary: Plastid establishment did not require a chlamydial partner. *Frontiers in Cellular and Infection Microbiology* **6**: 43.

Ball SG, Bhattacharya D, Weber AP. 2016b. Infection and the first eukaryotes - response. *Science* **352**: 1065-1066.

Ball SG, Bhattacharya D, Weber AP. 2016c. Pathogen to powerhouse. *Science* **351**: 659-660.

Ball SG, Subtil A, Bhattacharya D, Moustafa A, Weber AP, Gehre L, Colleoni C, Arias MC, Cenci U, Dauvillee D. 2013. Metabolic effectors secreted by bacterial pathogens: essential facilitators of plastid endosymbiosis? *Plant Cell* **25**: 7-21.

Barott KL, Venn AA, Perez SO, Tambutte S, Tresguerres M. 2015. Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 607-612.

Becker B, Hoef-Emden K, Melkonian M. 2008. Chlamydial genes shed light on the evolution of photoautotrophic eukaryotes. *BMC Evolutionary Biology* **8**: 203.

Behrenfeld MJ, O'Malley RT, Siegel DA, McClain CR, Sarmiento JL, Feldman GC, Milligan AJ, Falkowski PG, Letelier RM, Boss ES. 2006. Climate-driven trends in contemporary ocean productivity. *Nature* **444**: 752-755.

Bell W, Mitchell R. 1972. Chemotactic and growth responses of marine bacteria to algal extracellular products. *Biological Bulletin* **143**: 265-277.

Bertrand EM, McCrow JP, Moustafa A, Zheng H, McQuaid JB, Delmont TO, Post AF, Sipler RE, Spackeen JL, Xu K et al. 2015. Phytoplankton-bacterial interactions mediate micronutrient colimitation at the coastal Antarctic sea ice edge. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 9938-9943.

Bhattacharya D, Agrawal S, Aranda M, Baumgarten S, Belcaid M, Drake JL, Erwin D, Forêt S, Gates RD, Gruber DF et al. 2016. Comparative genomics explains the evolutionary success of reef-forming corals. *eLife* **5**: e13288.

Bhattacharya D, Yoon HS, Hackett JD. 2004. Photosynthetic eukaryotes unite: endosymbiosis connects the dots. *BioEssays* **26**: 50-60.

Bidle KD. 2015. The molecular ecophysiology of programmed cell death in marine phytoplankton. *Annual Review of Marine Science* **7**: 341-375.

Bidle KD, Haramaty L, Barcelos e Ramos J, Falkowski P. 2007. Viral activation and recruitment of metacaspases in the unicellular coccolithophore, *Emiliania huxleyi*.

Proceedings of the National Academy of Sciences of the United States of America **104**: 6049-6054.

Bonner CA, Byrne GI, Jensen RA. 2014. Chlamydia exploit the mammalian tryptophan-depletion defense strategy as a counter-defensive cue to trigger a survival state of persistence. *Frontiers in Cellular and Infection Microbiology* **4**: 17.

Bordenstein SR, Theis KR. 2015. Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biology* **13**: e1002226.

Bork P, Bowler C, de Vargas C, Gorsky G, Karsenti E, Wincker P. 2015. *Tara* Oceans studies plankton at planetary scale. *Science* **348**: 873.

Boucias DG, Kariithi HM, Bourtzis K, Schneider DI, Kelley K, Miller WJ, Parker AG, Abd-Alla AM. 2013. Transgenerational transmission of the *Glossina pallidipes* hytrosavirus depends on the presence of a functional symbiome. *PLoS ONE* **8**: e61150.

Brum JR, Sullivan MB. 2015. Rising to the challenge: accelerated pace of discovery transforms marine virology. *Nature Reviews Microbiology* **13**: 147-159.

Cavalier-Smith T. 1982. The origins of plastids. *Biological Journal of the Linnean Society* **17**: 289-306.

Cavalier-Smith T. 2002. The phagotrophic origin of eukaryotes and phylogenetic classification of protozoa. *International Journal of Systematic and Evolutionary Microbiology* **52**: 297-354.

Cenci U, Bhattacharya D, Weber AP, Colleoni C, Subtil A, Ball SG. 2017. Biotic host-pathogen interactions as major drivers of plastid endosymbiosis. *Trends in Plant Science* **22**: 316-328.

Cenci U, Ducatez M, Kadouche D, Colleoni C, Ball SG. 2016. Was the chlamydial adaptative strategy to tryptophan starvation an early determinant of plastid endosymbiosis? *Frontiers in Cellular and Infection Microbiology* **6**: 67.

Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG. 2005. Algae acquire vitamin B_{12} through a symbiotic relationship with bacteria. *Nature* **438**: 90-93.

Dagan T, Roettger M, Stucken K, Landan G, Koch R, Major P, Gould SB, Goremykin VV, Rippka R, Tandeau de Marsac N et al. 2013. Genomes of Stigonematalean cyanobacteria (subsection V) and the evolution of oxygenic photosynthesis from prokaryotes to plastids. *Genome Biology and Evolution* **5**: 31-44.

Delaye L, Valadez-Cano C, Pérez-Zamorano B 2016. How really ancient is *Paulinella chromatophora*? *PLoS Currents Tree of Life* doi:10.1371/currents.tol.e68a099364bb1a1e129a17b4e06b0c6b.

Deschamps P. 2014. Primary endosymbiosis: have cyanobacteria and Chlamydiae ever been roommates? *Acta Societatis Botanicorum Poloniae* **83**: 291-302.

Dittami SM, Duboscq-Bidot L, Perennou M, Gobet A, Corre E, Boyen C, Tonon T. 2016. Host-microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. *ISME Journal* **10**: 51-63.

Douglas AE, Werren JH. 2016. Holes in the hologenome: why host-microbe symbioses are not holobionts. *MBio* **7**: e02099.

Durham BP, Sharma S, Luo H, Smith CB, Amin SA, Bender SJ, Dearth SP, Van Mooy BA, Campagna SR, Kujawinski EB et al. 2015. Cryptic carbon and sulfur cycling between surface ocean plankton. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 453-457.

Emelyanov VV. 2003. Mitochondrial connection to the origin of the eukaryotic cell. *European Journal of Biochemistry* **270**: 1599-1618.

Enav H, Mandel-Gutfreund Y, Béjà O. 2014. Comparative metagenomic analyses reveal viral-induced shifts of host metabolism towards nucleotide biosynthesis. *Microbiome* **2**: 9.

Facchinelli F, Weber AP. 2011. The metabolite transporters of the plastid envelope: an update. *Frontiers in Plant Science* **2**: 50.

FAO. 2014. *The State of World Fisheries and Aquaculture*. Rome: Food and Agriculture Organization of the United Nations.

Felsenstein J. 1974. The evolutionary advantage of recombination. *Genetics* **78**: 737-756.

Gehre L, Gorgette O, Perrinet S, Prevost MC, Ducatez M, Giebel AM, Nelson DE, Ball SG, Subtil A. 2016. Sequestration of host metabolism by an intracellular pathogen. *eLife* **5**: e12552.

Gobler CJ, Norman C, Panzeca C, Taylor GT, Sañudo-Wilhelmy SA. 2007. Effect of B-vitamins (B_1, B_{12}) and inorganic nutrients on algal bloom dynamics in a coastal ecosystem. *Aquatic Microbial Ecology* **49**: 181-194.

Gould SB, Waller RF, McFadden GI. 2008. Plastid evolution. *Annual Review of Plant Biology* **59**: 491-517.

Helliwell KE, Lawrence AD, Holzer A, Kudahl UJ, Sasso S, Krautler B, Scanlan DJ, Warren MJ, Smith AG. 2016. Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B_{12}. *Current Biology* **26**: 999-1008.

Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research* **50**: 839-866.

Hopes A, Nekrasov V, Kamoun S, Mock T. 2016. Editing of the urease gene by CRISPR-Cas in the diatom *Thalassiosira pseudonana*. *Plant Methods* **12**: 49.

Howe CJ, Nisbet RE, Barbrook AC. 2008. The remarkable chloroplast genome of dinoflagellates. *Journal of Experimental Botany* **59**: 1035-1045.

Huang JL, Gogarten JP. 2007. Did an ancient chlamydial endosymbiosis facilitate the establishment of primary plastids? *Genome Biology* **8**: R99.

Jackson CJ, Waller RF. 2013. A widespread and unusual RNA trans-splicing type in dinoflagellate mitochondria. *PLoS ONE* **8**: e56777.

Karkar S, Facchinelli F, Price DC, Weber AP, Bhattacharya D. 2015. Metabolic connectivity as a driver of host and endosymbiont integration. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 10208-10215.

Kazamia E, Smith AG. 2014. Assessing the environmental sustainability of biofuels. *Trends in Plant Science* **19**: 615-618.

Korpinen S, Honkanen T, Vesakoski O, Hemmi A, Koivikko R, Loponen J, Jormalainen V. 2007. Macroalgal communities face the challenge of changing biotic interactions: review with focus on the Baltic Sea. *Ambio* **36**: 203-211.

Kostygov AY, Dobáková E, Grybchuk-Ieremenko A, Váhala D, Maslov DA, Votýpka J, Lukeš J, Yurchenko V. 2016. Novel trypanosomatid-bacterium association: evolution of endosymbiosis in action. *MBio* **7**: e01985.

LaJeunesse TC, Lambert G, Andersen RA, Coffroth MA, Galbraith DW. 2005. *Symbiodinium* (Pyrrhophyta) genome sizes (DNA content) are smallest among dinoflagellates. *Journal of Phycology* **41**: 880-886.

Leggat W, Rees TA, Yellowlees D. 2000. Meeting the photosynthetic demand for inorganic carbon in an alga-invertebrate association: preferential use of CO_2 by symbionts in the giant clam *Tridacna gigas*. *Proceedings of the Royal Society B: Biological Sciences* **267**: 523-529.

Lehahn Y, Koren I, Schatz D, Frada M, Sheyn U, Boss E, Efrati S, Rudich Y, Trainic M, Sharoni S et al. 2014. Decoupling physical from biological processes to assess the impact of viruses on a mesoscale algal bloom. *Current Biology* **24**: 2041-2046.

Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, Chaffron S, Ignacio-Espinosa JC, Roux S, Vincent F et al. 2015. Ocean plankton. Determinants of community structure in the global plankton interactome. *Science* **348**: 1262073.

Lin S. 2011. Genomic understanding of dinoflagellates. *Research in Microbiology* **162**: 551-569.

Lin S, Cheng S, Song B, Zhong X, Lin X, Li W, Li L, Zhang Y, Zhang H, Ji Z et al. 2015. The *Symbiodinium kawagutii* genome illuminates dinoflagellate gene expression and coral symbiosis. *Science* **350**: 691-694.

Malitsky S, Ziv C, Rosenwasser S, Zheng S, Schatz D, Porat Z, Ben-Dor S, Aharoni A, Vardi A. 2016. Viral infection of the marine alga *Emiliania huxleyi* triggers lipidome remodeling and induces the production of highly saturated triacylglycerol. *New Phytologist* **210**: 88-96.

Marin B, Nowack ECM, Melkonian M. 2005. A plastid in the making: evidence for a second primary endosymbiosis. *Protist* **156**: 425-432.

Martijn J, Schulz F, Zaremba-Niedzwiedzka K, Viklund J, Stepanauskas R, Andersson SG, Horn M, Guy L, Ettema TJ. 2015. Single-cell genomics of a rare environmental alphaproteobacterium provides unique insights into Rickettsiaceae evolution. *ISME Journal* **9**: 2373-2385.

Martin W, Herrmann RG. 1998. Gene transfer from organelles to the nucleus: how much, what happens, and why? *Plant Physiology* **118**: 9-17.

McEwan M, Humayun R, Slamovits CH, Keeling PJ. 2008. Nuclear genome sequence survey of the dinoflagellate *Heterocapsa triquetra*. *Journal of Eukaryotic Microbiology* **55**: 530-535.

McRose D, Guo J, Monier A, Sudek S, Wilken S, Yan S, Mock T, Archibald JM, Begley TP, Reyes-Prieto A et al. 2014. Alternatives to vitamin B_1 uptake revealed with discovery of riboswitches in multiple marine eukaryotic lineages. *ISME Journal* **8**: 2517-2529.

Mohamed AR, Cumbo V, Harii S, Shinzato C, Chan CX, Ragan MA, Bourne DG, Willis BL, Ball EE, Satoh N et al. 2016. The transcriptomic response of the coral *Acropora digitifera* to a competent *Symbiodinium* strain: the symbiosome as an arrested early phagosome. *Molecular Ecology* **25**: 3127-3141.

Moniruzzaman M, Gann ER, LeCleir GR, Kang Y, Gobler CJ, Wilhelm SW. 2016. Diversity and dynamics of algal Megaviridae members during a harmful brown tide caused by the pelagophyte, *Aureococcus anophagefferens*. *FEMS Microbiology Ecology* **92**: fiw058.

Monteiro FM, Bach LT, Brownlee C, Bown P, Rickaby RE, Poulton AJ, Tyrrell T, Beaufort L, Dutkiewicz S, Gibbs S et al. 2016. Why marine phytoplankton calcify. *Science Advances* **2**: e1501822.

Moog D, Rensing SA, Archibald JM, Maier UG, Ullrich KK. 2015. Localization and evolution of putative triose phosphate translocators in the diatom *Phaeodactylum tricornutum*. *Genome Biology and Evolution* **7**: 2955-2969.

Morales J, Kokkori S, Weidauer D, Chapman J, Goltsman E, Rokhsar D, Grossman AR, Nowack EC. 2016. Development of a toolbox to dissect host-endosymbiont interactions and protein trafficking in the trypanosomatid *Angomonas deanei*. *BMC Evolutionary Biology* **16**: 247.

Moustafa A, Reyes-Prieto A, Bhattacharya D. 2008. Chlamydiae has contributed at least 55 genes to Plantae with predominantly plastid functions. *PLoS ONE* **3**: e2205.

Mungpakdee S, Shinzato C, Takeuchi T, Kawashima T, Koyanagi R, Hisata K, Tanaka M, Goto H, Fujie M, Lin S et al. 2014. Massive gene transfer and extensive RNA editing of a symbiotic dinoflagellate plastid genome. *Genome Biology and Evolution* **6**: 1408-1422.

Muscatine L, Porter JW. 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* **27**: 454-460.

Nakayama T, Kamikawa R, Tanifuji G, Kashiyama Y, Ohkouchi N, Archibald JM, Inagaki Y. 2014. Complete genome of a nonphotosynthetic cyanobacterium in a diatom reveals recent adaptations to an intracellular lifestyle. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 11407-11412.

Needham DM, Fuhrman JA. 2016. Pronounced daily succession of phytoplankton, archaea and bacteria following a spring bloom. *Nature Microbiology* **1**: 16005.

Nowack EC, Grossman AR. 2012. Trafficking of protein into the recently established photosynthetic organelles of *Paulinella chromatophora*. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 5340-5345.

Nowack ECM, Melkonian M, Glockner G. 2008. Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Current Biology* **18**: 410-418.

Nowack ECM, Price DC, Bhattacharya D, Singer A, Melkonian M, Grossman AR. 2016. Gene transfers from diverse bacteria compensate for reductive genome evolution in the chromatophore of *Paulinella chromatophora*. *Proceedings of the National Academy of Sciences of the United States of America* **113**: 12214-12219.

Nymark M, Sharma AK, Sparstad T, Bones AM, Winge P. 2016. A CRISPR/Cas9 system adapted for gene editing in marine algae. *Scientific Reports* **6**: 24951.

Paerl RW, Bertrand EM, Allen AE, Palenik B, Azam F. 2015. Vitamin B1 ecophysiology of marine picoeukaryotic algae: strain-specific differences and a new role for bacteria in vitamin cycling. *Limnology and Oceanography* **60**: 215-228.

Palmer JD. 2003. The symbiotic birth and spread of plastids: how many times and whodunit? *Journal of Phycology* **39**: 4-11.

Petitpas CM, Turner JT, Deeds JR, Keafer BA, McGillicuddy DJ, Jr., Milligan PJ, Shue V, White KD, Anderson DM. 2014. PSP toxin levels and plankton community composition

and abundance in size-fractionated vertical profiles during spring/summer blooms of the toxic dinoflagellate *Alexandrium fundyense* in the Gulf of Maine and on Georges Bank, 2007, 2008, and 2010: 2. Plankton community composition and abundance. *Deep Sea Research Part II: Topical Studies in Oceanogrpahy* **103**: 350-367.

Price DC, Chan CX, Yoon HS, Yang EC, Qiu H, Weber AP, Schwacke R, Gross J, Blouin NA, Lane C et al. 2012. *Cyanophora paradoxa* genome elucidates origin of photosynthesis in algae and plants. *Science* **335**: 843-847.

Qiu D, Huang L, Lin S. 2016. Cryptophyte farming by symbiotic ciliate host detected in situ. *Proceedings of the National Academy of Sciences of the United States of America* **113**: 12208-12213.

Qiu H, Price DC, Weber APM, Facchinelli F, Yoon HS, Bhattacharya D. 2013. Assessing the bacterial contribution to the plastid proteome. *Trends in Plant Science* **18**: 680-687.

Radecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C. 2015. Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends in Microbiology* **23**: 490-497.

Raven JA. 2015. Implications of mutation of organelle genomes for organelle function and evolution. *Journal of Experimental Botany* **66**: 5639-5650.

Read BA, Kegel J, Klute MJ, Kuo A, Lefebvre SC, Maumus F, Mayer C, Miller J, Monier A, Salamov A et al. 2013. Pan genome of the phytoplankton *Emiliania* underpins its global distribution. *Nature* **499**: 209-213.

Roossinck MJ. 1997. Mechanisms of plant virus evolution. *Annual Review of Phytopathology* **35**: 191-209.

Rosenwasser S, Mausz MA, Schatz D, Sheyn U, Malitsky S, Aharoni A, Weinstock E, Tzfadia O, Ben-Dor S, Feldmesser E et al. 2014. Rewiring host lipid metabolism by large viruses determines the fate of *Emiliania huxleyi*, a bloom-forming alga in the ocean. *Plant Cell* **26**: 2689-2707.

Rosenwasser S, Ziv C, Creveld SG, Vardi A. 2016. Virocell metabolism: metabolic innovations during host-virus interactions in the ocean. *Trends in Microbiology* **24**: 821-832.

Roth E, Jeon K, Stacey G 1988. Homology in endosymbiotic systems: the term 'symbiosome'. In: Palacious R, Verma DPS eds. *Molecular Genetics of Plant-Microbe Interactions*. St Paul, MN: American Phytopathology Society, 220-225.

Sañudo-Wilhelmy SA, Cutter LS, Durazo R, Smail EA, Gomez-Consarnau L, Webb EA, Prokopenko MG, Berelson WM, Karl DM. 2012. Multiple B-vitamin depletion in large areas of the coastal ocean. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 14041-14045.

Schleiff E, Becker T. 2011. Common ground for protein translocation: access control for mitochondria and chloroplasts. *Nature Reviews Molecular Cell Biology* **12**: 48-59.

Schroeder DC, Oke J, Malin G, Wilson WH. 2002. Coccolithovirus (Phycodnaviridae): characterisation of a new large dsDNA algal virus that infects *Emiliana huxleyi*. *Archives of Virology* **147**: 1685-1698.

Segev E, Wyche TP, Kim KH, Petersen J, Ellebrandt C, Vlamakis H, Barteneva N, Paulson JN, Chai L, Clardy J et al. 2016. Dynamic metabolic exchange governs a marine algal-bacterial interaction. *eLife* **5**.

Seyedsayamdost MR, Case RJ, Kolter R, Clardy J. 2011. The Jekyll-and-Hyde chemistry of *Phaeobacter gallaeciensis*. *Nature Chemistry* **3**: 331-335.

Shapiro OH, Kramarsky-Winter E, Gavish AR, Stocker R, Vardi A. 2016. A coral-on-a-chip microfluidic platform enabling live-imaging microscopy of reef-building corals. *Nature Communications* **7**: 10860.

Sheyn U, Rosenwasser S, Ben-Dor S, Porat Z, Vardi A. 2016. Modulation of host ROS metabolism is essential for viral infection of a bloom-forming coccolithophore in the ocean. *ISME Journal* **10**: 1742-1754.

Shinzato C, Mungpakdee S, Satoh N, Shoguchi E. 2014. A genomic approach to coral-dinoflagellate symbiosis: studies of *Acropora digitifera* and *Symbiodinium minutum*. *Frontiers in Microbiology* **5**: 336.

Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujiwara M, Koyanagi R, Ikuta T et al. 2011. Using the *Acropora digitifera* genome to understand coral responses to environmental change. *Nature* **476**: 320-323.

Shoguchi E, Shinzato C, Kawashima T, Gyoja F, Mungpakdee S, Koyanagi R, Takeuchi T, Hisata K, Tanaka M, Fujiwara M et al. 2013. Draft assembly of the *Symbiodinium minutum* nuclear genome reveals dinoflagellate gene structure. *Current Biology* **23**: 1399-1408.

Silverstein RN, Correa AM, Baker AC. 2012. Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. *Proceedings of the Royal Society B: Biological Sciences* **279**: 2609-2618.

Spang A, Saw JH, Jorgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, Schleper C, Guy L, Ettema TJ. 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* **521**: 173-179.

Spoerner M, Wichard T, Bachhuber T, Stratmann J, Oertel W. 2012. Growth and thallus morphogenesis of *Ulva mutabilis* (Chlorophyta) depends on a combination of two bacterial species excreting regulatory factors. *Journal of Phycology* **48**: 1433-1447.

Suttle CA. 2007. Marine viruses—major players in the global ecosystem. *Nature Reviews Microbiology* **5**: 801-812.

Tripp EA, Zhang N, Schneider H, Huang Y, Mueller GM, Hu Z, Haggblom M, Bhattacharya D 2017. Reshaping Darwin's tree: impact of the symbiome. *Trends in Ecology & Evolution* doi:10.1016/j.tree.2017.05.002.

Tyrrell T, Merico A 2004. *Emiliania huxleyi*: bloom observations and the conditions that induce them. In: Thierstein HR, Young JR eds. *Coccolithophores: From Molecular Processes to Global Impact*. Berlin, Heidelberg: Springer, 75-97.

van Creveld SG, Rosenwasser S, Schatz D, Koren I, Vardi A. 2015. Early perturbation in mitochondria redox homeostasis in response to environmental stress predicts cell fate in diatoms. *ISME Journal* **9**: 385-395.

Vardi A, Haramaty L, Van Mooy BA, Fredricks HF, Kimmance SA, Larsen A, Bidle KD. 2012. Host-virus dynamics and subcellular controls of cell fate in a natural coccolithophore population. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 19327-19332.

Vardi A, Van Mooy BA, Fredricks HF, Popendorf KJ, Ossolinski JE, Haramaty L, Bidle KD. 2009. Viral glycosphingolipids induce lytic infection and cell death in marine phytoplankton. *Science* **326**: 861-865.

Voolstra C, Miller D, Ragan M, Hoffmann A, Hoegh-Guldberg O, Bourne D, Ball E, Ying H, Foret S, Takahashi S et al. 2015. The ReFuGe 2020 Consortium—using "omics" approaches to explore the adaptability and resilience of coral holobionts to environmental change. *Frontiers in Marine Science* **2**: 68.

Wagner-Döbler I, Ballhausen B, Berger M, Brinkhoff T, Buchholz I, Bunk B, Cypionka H, Daniel R, Drepper T, Gerdts G et al. 2010. The complete genome sequence of the algal symbiont *Dinoroseobacter shibae*: a hitchhiker's guide to life in the sea. *ISME Journal* **4**: 61-77.

Wahl M, Goecke F, Labes A, Dobretsov S, Weinberger F. 2012. The second skin: ecological role of epibiotic biofilms on marine organisms. *Frontiers in Microbiology* **3**: 292.

Walker NA, Smith FA, Cathers IR. 1980. Bicarbonate assimilation by fresh-water charophytes and higher plants: I. Membrane transport of bicarbonate ions is not proven. *The Journal of Membrane Biology* **57**: 51-58.

Wang Z, Wu M. 2015. An integrated phylogenomic approach toward pinpointing the origin of mitochondria. *Scientific Reports* **5**: 7949.

Warren MJ, Raux E, Schubert HL, Escalante-Semerena JC. 2002. The biosynthesis of adenosylcobalamin (vitamin B_{12}). *Natural Product Reports* **19**: 390-412.

Wham DC, LaJeunesse TC. 2016. *Symbiodinium* population genetics: testing for species boundaries and analysing samples with mixed genotypes. *Molecular Ecology* **25**: 2699-2712.

Wilkinson C. 2004. *Status of Coral Reefs of the World: 2004*. Townsville, QLD: Australian Institute of Marine Science.

Wilson WH, Schroeder DC, Allen MJ, Holden MT, Parkhill J, Barrell BG, Churcher C, Hamlin N, Mungall K, Norbertczak H et al. 2005. Complete genome sequence and lytic phase transcription profile of a Coccolithovirus. *Science* **309**: 1090-1092.

Wilson WH, Van Etten JL, Allen MJ. 2009. The Phycodnaviridae: the story of how tiny giants rule the world. *Current Topics in Microbiology and Immunology* **328**: 1-42.

Wooldridge SA. 2013. Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. *Biogeosciences* **10**: 1647-1658.

Worden AZ, Follows MJ, Giovannoni SJ, Wilken S, Zimmerman AE, Keeling PJ. 2015. Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. *Science* **347**: 1257594.

Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. 2004. A molecular timeline for the origin of photosynthetic eukaryotes. *Molecular Biology and Evolution* **21**: 809-818.

Yoon HS, Nakayama T, Reyes-Prieto A, Andersen RA, Boo SM, Ishida K, Bhattacharya D. 2009. A single origin of the photosynthetic organelle in different *Paulinella* lineages. *BMC Evolutionary Biology* **9**: 98.

Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU et al. 2017. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* **541**: 353-358.

Zehr JP, Shilova IN, Farnelid HM, Muñoz-Marin MC, Turk-Kubo KA. 2016. Unusual marine unicellular symbiosis with the nitrogen-fixing cyanobacterium UCYN-A. *Nature Microbiology* **2**: 16214.

Zhang H, Hou Y, Miranda L, Campbell DA, Sturm NR, Gaasterland T, Lin S. 2007. Spliced leader RNA trans-splicing in dinoflagellates. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 4618-4623.

Zhang Z, Green BR, Cavalier-Smith T. 1999. Single gene circles in dinoflagellate chloroplast genomes. *Nature* **400**: 155-159.

Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR. 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nature Communications* **8**: 14213.

Ziv C, Malitsky S, Othman A, Ben-Dor S, Wei Y, Zheng S, Aharoni A, Hornemann T, Vardi A. 2016. Viral serine palmitoyltransferase induces metabolic switch in sphingolipid biosynthesis and is required for infection of a marine alga. *Proceedings of the National Academy of Sciences of the United States of America* **113**: E1907-E1916.

Zomorodipour A, Andersson SG. 1999. Obligate intracellular parasites: *Rickettsia prowazekii* and *Chlamydia trachomatis*. *FEBS Letters* **452**: 11-15.

Figure legends

Fig. 1 Evolutionary history of algae. a) Schematic tree of eukaryotes showing the polyphyletic origins of algae. Plastids derived through primary cyanobacterial endosymbiosis are shown in blue text and when derived through secondary or higher order endosymbioses are shown in brown text. The major clades are often referred to as supergroups, except the orphan algae that do not yet have a stable position in molecular phylogenies. b) The ménage à trois hypothesis (MATH) for primary plastid origin in the Archaeplastida ancestor. The MATH proposes that environmental Chlamydiales played a direct role in plastid endosymbiosis vis-à-vis a tripartite relationship between the host, captured cyanobacterium, and a chlamydial symbiont (Ball *et al.*, 2013; Ball *et al.*, 2016a). Under this view, a chlamydial infectious particle (EB: elementary body, black circle) enters a host cell together with a cyanobacterium (turquoise circle). The EB remodels the phagocytic membrane into a chlamydia-controlled inclusion, thereby escaping host defenses. The EB differentiates into reticulate bodies (RBs; pink circles) that attach to the inclusion and secrete chlamydial effector proteins corresponding to glycogen metabolism enzymes into both the inclusion and the host cytosol. The cyanobacterium recruits chlamydial transporters through conjugation with Chlamydiae that allow the export of glucose-6-phosphate (G6P) through the UhpC transporter (yellow circle in the cyanobacterial cell envelopes). The G6P feeds glycogen synthesis within the inclusion through the ADP-G dependent chlamydial pathway of glycogen metabolism. Excess ADP-G in the inclusion exits through a host derived NST (nucleotide sugar transporter, red circle) and is incorporated into the host glycogen pool. This hypothetical sequence of steps is believed to have led to the establishment of the long-term symbiosis.

Fig. 2 Dinoflagellate symbionts in corals. a) *Acropora millepora* and b) *A. tenuis* showing tentacles associated with individual coral polyps and tissue color (with individual cells visible in *A. tenuis*) associated with a high abundance of *Symbiodinium* within the coral gastrodermis tissue layers (photo credits: Jean-Baptiste Raina). c) Metabolic exchange and nutrient trafficking between the coral animal and its *Symbiodinium* symbionts and extracellular microbes.

Fig. 3 Genome-enabled technologies can define distinct metabolic state of life cycle states during host-pathogen/symbiont interactions. The metabolic state of diverse life cycle states during host-

pathogen/symbiont interactions can be defined by combined high-throughput approaches including transcriptomics, proteomics, and metabolomics. Dimension reduction analyses such as principal component analysis (PCA) can be used to define the specific metabolic states of the algal host, its pathogen or free-living symbiont, and the unique metabolism of infected algae. Such characterization provides metabolic fingerprints that can serve as novel biomarkers to assess host-pathogen interactions in the marine environment, and to unravel the strategies employed during biochemical "arms race" of host-pathogen co-evolution. Figure modified from Rosenwasser *et al*. (2016).

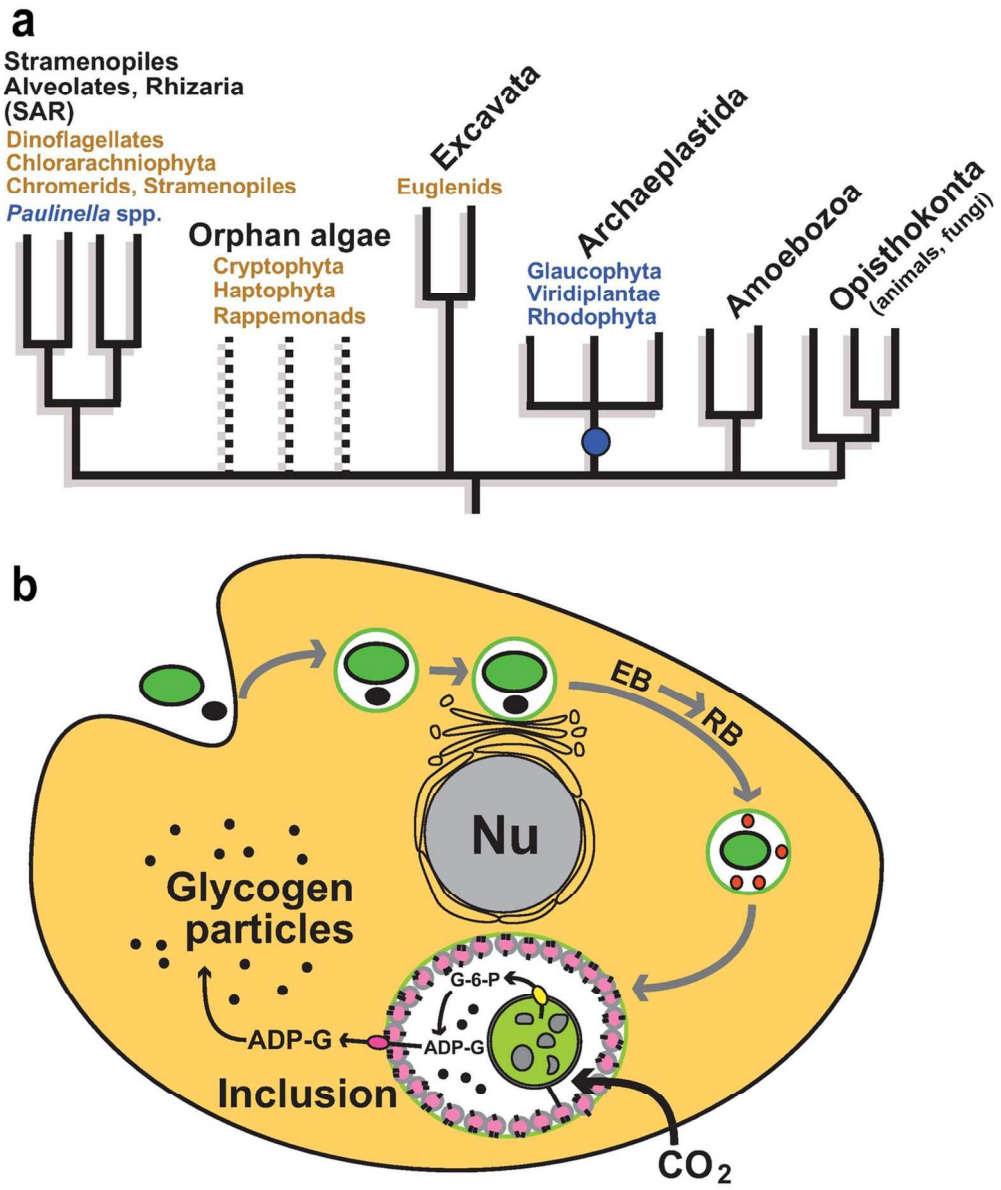

Fig. 1 Evolutionary history of algae. a) Schematic tree of eukaryotes showing the polyphyletic origins of algae. Plastids derived through primary cyanobacterial endosymbiosis are shown in blue text and when derived through secondary or higher order endosymbioses are shown in brown text. The major clades are often referred to as supergroups, except the orphan algae that do not yet have a stable position in molecular phylogenies. b) The ménage à trois hypothesis (MATH) for primary plastid origin in the Archaeplastida ancestor. The MATH proposes that environmental Chlamydiales played a direct role in plastid endosymbiosis vis-à-vis a tripartite relationship between the host, captured cyanobacterium, and a chlamydial symbiont (Ball et al., 2013; Ball et al., 2016a). Under this view, a chlamydial infectious particle (EB: elementary body, black circle) enters a host cell together with a cyanobacterium (turquoise circle). The EB remodels the phagocytic membrane into a chlamydia-controlled inclusion, thereby escaping host defenses. The EB differentiates into reticulate bodies (RBs; pink circles) that attach to the inclusion and secrete chlamydial effector proteins corresponding to glycogen metabolism enzymes into both the inclusion and the host cytosol. The cyanobacterium recruits chlamydial transporters through conjugation with

Chlamydiae that allow the export of glucose-6-phosphate (G6P) through the UhpC transporter (yellow circle in the cyanobacterial cell envelopes). The G6P feeds glycogen synthesis within the inclusion through the ADP-G dependent chlamydial pathway of glycogen metabolism. Excess ADP-G in the inclusion exits through a host derived NST (nucleotide sugar transporter, red circle) and is incorporated into the host glycogen pool. This hypothetical sequence of steps is believed to have led to the establishment of the long-term symbiosis.

112x135mm (300 x 300 DPI)

Fig. 2 Dinoflagellate symbionts in corals. a) Acropora millepora and b) A. tenuis showing tentacles associated with individual coral polyps and tissue color (with individual cells visible in A. tenuis) associated with a high abundance of Symbiodinium within the coral gastrodermis tissue layers (photo credits: Jean-Baptiste Raina). c) Metabolic exchange and nutrient trafficking between the coral animal and its Symbiodinium symbionts and extracellular microbes.

61x22mm (300 x 300 DPI)

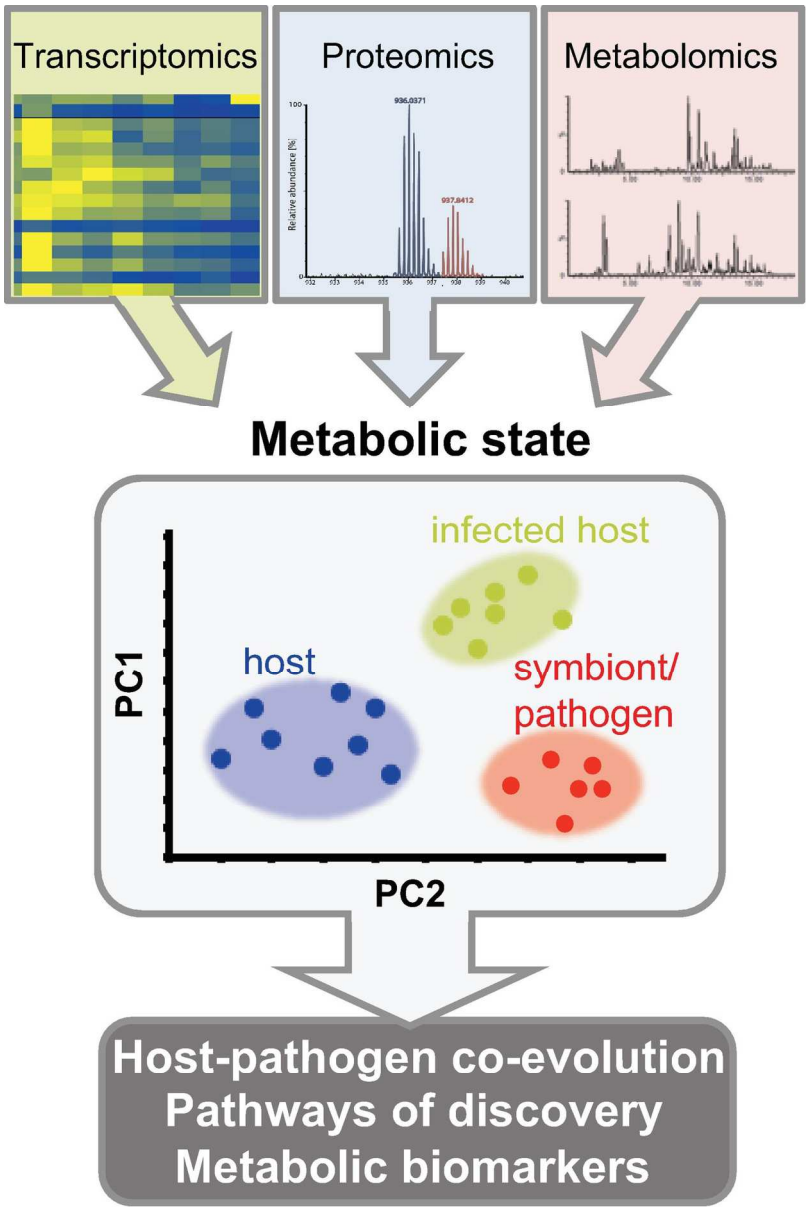

Fig. 3 Genome-enabled technologies can define distinct metabolic state of life cycle states during host-pathogen/symbiont interactions. The metabolic state of diverse life cycle states during host-pathogen/symbiont interactions can be defined by combined high-throughput approaches including transcriptomics, proteomics, and metabolomics. Dimension reduction analyses such as principal component analysis (PCA) can be used to define the specific metabolic states of the algal host, its pathogen or free-living symbiont, and the unique metabolism of infected algae. Such characterization provides metabolic fingerprints that can serve as novel biomarkers to assess host-pathogen interactions in the marine environment, and to unravel the strategies employed during biochemical "arms race" of host-pathogen co-evolution. Figure modified from Rosenwasser et al. (2016).

125x187mm (300 x 300 DPI)